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STUDIES ON THE COMPATIBILITY OF BACTERIAL ANTAGONISTS WITH COMMON AGROCHEMICALS AGAINST *XANTHOMONAS ORYZAE* pv. *ORYZAE*, THE RICE BACTERIAL LEAF BLIGHT PATHOGEN

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Abstract: Seven bacterial antagonistic bacteria *viz*., RE-1, RR-26, RR-53, CB-39, VB-67, VB-69 and Pf-1 were subjected to compatibility studies against the rice bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) under *in vitro*. The study revealed that except the two combinations *viz*., VB-67 with VB-69 and VB-67 with CB-39, the rest of the 17 combinations showed synergistic effect in inhibiting *Xoo*. The compatibility of seven antagonists were tested with nine pesticides *viz*., chlorpyriphos, dimethoate, triazophos, quinalphos, dichlorvos, carbendazim, mancozeb, propiconazole and hexaconazole and with four fertilizers *viz*., urea, rajphos, muriate of potash and ammonium sulphate under *in vitro* against *Xoo*. Out of 91 two way combinations tried, 71 two way combinations were found having synergestic effect against *Xoo* and the rest 12 combinations *viz*., RE-1 with dimethoate, VB-69 with dichlorvos, RR-53 with triazophos, Pf1 with carbendazim , VB-69 with hexaconazole, RR-53 with hexaconazole, RR-26 with rajphos, RR-26 with muriate of potash, RR-26 with ammonium sulphate, CB-39 with ammonium sulphate and RR-53 with rajphos showed non compatible effect against *Xoo*. The compatibility of thirteen agrochemicals *viz*., chlorpyriphos, dimethoate, triazophos, quinalphos, dichlorvos, carbendazim, mancozeb, propiconazole, hexaconazole, urea, rajphos, muriate of potash and ammonium sulphate with nine pesticides *viz*., chlorpyriphos, dimethoate, triazophos, quinalphos, dichlorvos, carbendazim, mancozeb, propiconazole and hexaconazole under *in vitro* against *Xoo*. The study revealed that out of 71 two way combinations tested, 50 two way combinations showed synergistic action in inhibiting the pathogen. Three two way combinations *viz*., quinalphos with dichlorvos, dichlorvos with urea and hexaconazole with ammonium sulphate showed compatible action against *Xoo* and the rest 19 combinations *viz*., chlorpyriphos with carbendazim, chlorpyriphos with urea, dimethoate with muriate of potash, dimethoate with ammonium sulphate, triazophos with propiconazloe, triazophos with urea, triazophos with muriate of potash, quinalphos with hexaconazole, quinalphos with urea, quinalphos with muriate of potash, dichlorvos with carbendazim, carbendazim with propiconazloe, carbendazim with hexaconazole, carbendazim with urea, carbendazim with rajphos, carbendazim with muriate of potash, carbendazim with ammonium sulphate, hexaconazole with urea and hexaconazole with rajphos showed non compatible effect against *Xoo*. The compatibility of four fertilizers *viz*., urea, rajphos, muriate of potash and ammonium sulphate showed that out of six two way combinations, only one namely urea with rajphos showed non compatible action in inhibiting the *Xoo* and the rest five two way combinations showed synergistic action in inhibiting the pathogen. All the seven antagonistic bacteria and 17 agrochemicals showed compatible reaction in inhibiting the *Xoo*.

Key words: Antagonistic bacteria, Pesticides, Fertilizers, Rice bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae*, Inhibition

INTRODUCTION

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most important diseases of rice and the disease was first noticed by the farmers of Japan in 1884 (Tagami and Mizukami, 1962). Severe crop losses of 10-20% in moderate conditions or up to 50% in highly conducive conditions have been recorded in several of Asian and South East Asian countries (Mew, 1987; Ou, 1985). Globally, its incidence has been reported from different parts of Asia, Northern Australia, Africa and USA. In India, BLB disease has been observed in most important rice-growing states like Andhra Pradesh, Bihar, Haryana, Kerala, Orissa, Punjab and Uttar Pradesh. The disease occurred in an epidemic form during 1998 in Palakkad district of Kerala (Venkatesan and Gnanamanickam, 1999) and

since then has been observed in severe proportions almost every year. Management of bacterial blight of rice has been carried out using chemicals and development of resistant cultivars over a long period of time. However, the application of chemicals is not always effective and may also affect the environment. Breeding for bacterial blight resistance is the most economic strategy of disease management and this has only been partially successful because of the enormous diversity in the pathogen. Recently, transgenic indica rices resistant to bacterial blight have been generated (Narayanan, 2001; Narayanan *et al.*, 2002) but are still to be field-evaluated in India.

Biological control, therefore, assumes special significance in being an ecology-conscious, cost-effective alternative strategy for bacterial blight management. This can also be used in integration with other strategies to afford greater levels of protection and sustain rice yields. Antagonistic bacteria are considered ideal biological control agents, due to reasons like rapid growth, easy handling and aggressive colonization of the rhizosphere (Weller, 1988).The beneficial effects of PGPR include direct growth promotion, biological control and inducing systemic resistance in host plants. PGPR strain mixtures have showed synergistic action in plant protection and growth promotion, indicating different mechanisms are involved in disease control. In this study, seven antagonistic bacteria were subjected among themselves and to different agrochemicals to study their compatibility against *Xoo* the rice bacterial leaf blight pathogen.

MATERIALS AND METHODS

While adopting integrated disease management practices using antagonists, it is necessary that the agrochemicals used in the fields should be compatible with the biocontrol agents and further care must be taken to select suitable combinations. With this in view, laboratory experiments were carried out to study the compatibility of bacterial antagonists with agrochemicals against *Xanthomonas oryzae pv.oryzae* (*Xoo*) the bacterial leaf blight of rice pathogen. Six antagonistic bacteria along with reference culture of KAU-(Pf1) and thirteen agrochemicals were taken for the study. The details of antagonists, pesticides and fertilizers used for this study are listed below:

No.	1. Antagonists
1.	RE-1 (Rice Endosphere bacteria)
2.	RR-26 (Rice Rhizosphere bacteria)
3.	RR-53 (Rice Rhizosphere bacteria)
4.	CB-39 (Cow dung bacteria)
5.	VB-67 (Vermicompost bacteria)
6.	VB-69 (Vermicompost bacteria)
7.	KAU-Pf1 (*Pseudomonas fluorescens*)

No.	2. Pesticides	Trade name
1.	Mancozeb	Indofil M-45
2.	Carbendazim	Bavistin-50 WP
3.	Propiconazole	Tilt-25 EC
4.	Hexaconazole	Contaf-5 EC
5.	Chlorpyriphos	Dursban-50 EC
6.	Dimethoate	Rogor-30 EC
7.	Dichlorvos	Nuvan-76 WSC
8.	Triazophos	Hostothion-40 EC
9.	Quinalphos	Ekalux-25 EC

No.	3. Fertilizers
1.	Urea (N- 46 %)
2.	Rajphos (P_2O_5 - 18-20%)
3.	Muriate of Potash (K_2O- 60%)
4.	Ammonium sulphate (N- 20.50%)

All of them were subjected to compatibility studies against *Xoo* under *in vitro* by paper disc method. All possible two way combinations were studied among themselves and with others.Concentration of different antagonistic bacteria was adjusted to10^8 cfu/ml. To prepare correct concentration of agrochemicals as per the Packages of Practices recommendations (POP) of Kerala Agricultural University, the actual quantity was mixed with 100 ml of sterile water and the stock solutions were made. Fertilizers were exposed to UV light for 45 minutes to avoid the contamination. Three discs were kept in each Petri dish dipped in two materials for studying the combination effect and the interaction between them. The discs dipped in single material were used to study the individual effect and they served as control also. The studies were carried out on PSA medium seeded with *Xoo*. Observations on zone of inhibition of *Xoo* were recorded after 48 h of incubation.

Table 1. Compatibility of bacterial antagonists with common agrochemicals against *Xoo*

Sl. No.	Bacterial antagonists& agrochemicals	RR-26	CB-39	RE-1	VB-67	VB-69	RR-53	Pf-1	Chlorpyriphos	Dimethoate	Triazophos	Quinalphos	Dichlorvos	Carbendazim	Mancozeb	Propiconazole	Hexacnazole	Urea	Rajphos	Muriate of potash	Ammonium sulphate
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	RR-26	2.50																			
2	CB-39	3.83	1.70																		
3	RE-1	1.13	3.86	3.00																	
4	VB-67	3.73	3.80	1.10	1.40																
5	VB-69	3.73	2.70	3.20	2.90	1.40															
6	RR-53	3.93	2.33	1.46	1.33	1.90	1.80														
7	Pf-1	1.36	1.46	3.36	1.20	2.86	1.56	2.50													
8	Chlorpyriphos	1.40	0.96	1.13	1.40	1.43	1.36	3.40	2.10												
9	Dimethoate	1.83	1.40	4.26	1.53	1.76	3.00	2.90	2.90	2.16											
10	Triazophos	1.03	1.46	1.30	1.70	2.33	4.00	3.46	3.46	4.00	2.10										
11	Quinalphos	2.66	1.06	2.86	1.13	1.20	0.90	2.96	2.96	3.83	1.66	2.03									
12	Dichlorvos	3.50	1.20	1.26	1.93	0.00	1.03	2.56	2.56	1.73	1.00	4.16	2.16								
13	Carbendazim	2.73	1.23	1.10	1.10	1.06	2.16	3.60	3.60	1.13	1.20	1.30	4.00	0.96							
14	Mancozeb	0.90	1.03	1.10	2.66	1.16	0.70	1.83	1.83	2.33	1.30	1.50	4.16	2.00	3.00						
15	Propiconazole	2.83	1.23	4.13	2.03	1.30	1.23	1.26	1.26	3.66	0.00	3.00	4.00	4.00	3.50	2.66					
16	Hexaconazole	1.46	0.86	1.53	1.63	0.00	0.00	0.86	0.86	2.23	3.16	4.16	1.83	4.00	4.00	2.83	1.33				
17	Urea	2.00	1.00	1.30	1.16	1.33	1.40	3.30	3.30	4.06	0.00	3.93	3.16	4.10	3.00	2.00	3.00	1.10			
18	Rajphos	3.70	1.03	1.33	1.43	1.70	4.50	2.06	2.06	2.33	2.00	1.60	2.00	3.50	3.00	3.16	3.16	3.16	0.73		
19	Muriate of potash	0.00	0.90	0.00	1.16	1.43	2.16	2.83	2.83	3.73	3.66	3.56	2.00	3.33	1.20	1.33	0.86	0.76	0.90	1.00	
20	Ammonium sulphate	3.60	0.00	1.60	1.20	1.76	1.36	1.80	1.80	3.66	2.00	1.90	1.00	3.00	2.50	2.16	2.16	0.86	1.00	0.90	0.83

Table 2. Compatibility of bacterial antagonists with common agrochemicals against *Xoo* Assumption Table (a+b)

Sl. No.	Bacterial antagonists& agrochemicals	RR-26	CB-39	RE-1	VB-67	VB-69	RR-53	Pf-1	Chlorpyriphos	Dimethoate	Triazophos	Quinalphos	Dichlorvos	Carbendazim	Mancozeb	Propiconazole	Hexacnazole	Urea	Rajphos	Muriate of potash	Ammonium sulphate
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	RR-26	2.50																			
2	CB-39	4.20	1.70																		
3	RE-1	5.50	4.70	3.00																	
4	VB-67	3.90	3.10	4.40	1.40																
5	VB-69	3.90	3.10	4.40	2.80	1.40															
6	RR-53	4.30	3.50	4.80	3.20	3.20	1.80														
7	Pf-1	5.00	4.20	5.50	3.90	3.90	4.30	2.50													
8	Chlorpyriphos	4.60	3.80	5.10	3.50	3.50	3.90	4.60	2.10												
9	Dimethoate	4.66	3.86	5.16	3.56	3.56	3.96	4.66	4.26	2.16											
10	Triazophos	4.60	3.80	5.10	3.50	3.50	3.90	4.60	4.20	4.26	2.10										
11	Quinalphos	4.53	3.73	5.03	3.43	3.43	3.83	4.53	4.13	4.19	4.13	2.03									
12	Dichlorvos	4.66	3.86	5.16	3.56	3.56	3.96	4.66	4.26	4.32	4.26	4.19	2.16								
13	Carbendazim	3.46	2.66	3.96	2.36	2.36	2.76	3.46	3.06	3.12	3.06	2.99	3.12	0.96							
14	Mancozeb	5.50	4.70	6.00	4.40	4.40	4.80	5.50	5.10	5.16	5.10	5.03	5.16	3.96	3.00						
15	Propiconazole	5.16	4.36	5.66	4.06	4.06	4.46	5.16	4.76	4.82	4.76	4.69	4.82	3.62	5.66	2.66					
16	Hexaconazole	3.83	3.03	4.33	2.73	2.73	3.13	3.83	3.43	3.49	3.43	3.36	3.49	2.29	4.33	3.99	1.33				
17	Urea	3.60	2.80	4.10	2.50	2.50	2.90	3.60	3.20	3.26	3.20	3.13	3.26	2.06	4.10	3.76	2.43	1.10			
18	Rajphos	3.23	2.43	3.73	2.13	2.13	2.53	3.23	2.83	2.89	2.83	2.76	2.89	1.69	3.73	3.39	2.06	1.83	0.73		
19	Muriate of potash	3.50	2.70	4.00	2.40	2.40	2.80	3.50	3.10	3.16	3.10	3.03	3.16	1.96	4.00	3.66	2.33	2.10	1.73	1.00	
20	Ammonium sulphate	3.33	2.53	3.83	2.23	2.23	2.63	3.33	2.93	2.99	2.93	2.86	2.99	1.79	3.83	3.49	2.16	1.93	1.56	1.83	0.83

RESULTS AND DISCUSSION

The compatibility of the six promising antagonistic bacteria *viz*., RE-1 (rice endosphere bacteria from Kodallur), RR-26 (rhizosphere bacteria from Nenmara), RR-53 (rhizosphere bacteria from Pattambi) CB-39 (bacteria from cow dung), VB-67 and VB-69 (bacteria from vermicompost) along with reference culture of KAU-(Pf1) with pesticides and fertilizers among themselves in two way combinations were studied and the results are presented in Table 1.

In the table, the inhibition presented as interaction of 1 with 1, 2 with 2, likewise represents, the individual effect of antagonist, pesticide or fertilizer as the case may be. It was considered as 'a' or 'b' as the study dealt with the individual and combined effect of these two materials. Therefore, 'a' and 'b' represent the individual effect (inhibition) on the pathogen. Likewise, the value in the interaction of 1 and 2 represents the combined effect of 1 and 2 and is represented as combined effect of 'a+b'. The data indicated that some combined effects were less than the half of additional value of 'a+b' (less than the individual inhibition effects) denoting that they were non compatible. In some cases, the combined effects were equal to the mathematical addition of a+b, indicating their interaction resulted in additive interaction, while some combinations resulted in the inhibition which was more than the additive effect of the component materials, suggesting a synergistic effect by their interaction. Some values showed that they are better than individual inhibition but not up to the level of addition, they are denoted as compatible. Hence, to interpret the data on compatibility and interaction among the study materials, the following calculations were made. The interaction values obtained are presented in the Table 2.

$$\text{Interaction value} = \frac{\text{Combined effect of (a + b)}}{\text{Addition of individual effect of a \& b}}$$

For easy understanding of the above table, the following notations are given and the table with 'non compatible', 'compatible', 'additive' and 'synergistic' interactions were made. An error component of 0.05 is allowed.

No	Notation	Description	Interaction value
1	NC	Non compatible	<0.5
2	C	Compatible	0.5to 1.0
3	A	Additive	1.0
4	S	Synergistic	>1.0

The compatibility of bacterial antagonists with agrochemicals against *Xoo* were studied and the results are presented in Table 3. 21 types of two way combinations among the seven antagonistic bacteria were studied for their compatibility against *Xoo*. Among them, two combinations *viz*., VB-67 with VB-69, VB-67 with CB-39 showed non compatible effect in inhibiting the *Xoo*. The rest of the 17 combinations showed synergistic effect in inhibiting the *Xoo*. Ramamoorthy *et al*. (2001) opined that, management of multiple pathogens and pests in crop plants can be achieved by applying mixture of strains showing synergistic action.

The compatibility of six antagonists *viz*., RE-1, RR-26, RR-53, CB-39, VB-67 and VB-69 along with the reference culture Pf1 were studied with nine pesticides *viz*., chlorpyriphos, dimethoate, quinalphos, dichlorvos, triazophos, carbendazim, mancozeb, propiconazole and hexaconazole and with four fertilizers *viz*., urea, rajphos, muriate of potash and ammonium sulphate under *in vitro* against *Xoo*. The study showed that, out of 91 two way combinations tried, 12 combinations *viz*., RE-1 with dimethoate, VB-69 with dichlorvos, RR-53 with triazophos, Pf1 with carbendazim, VB-69 with hexaconazole, RR-53 with hexaconazole, RR-26 with rajphos, RR-26 with muriate of potash, RR-26 with ammonium sulphate, CB-39 with ammonium sulphate and RR-53 with rajphos showed non compatible effect against *Xoo* and the rest showed synergestic effect against *Xoo*. The compatability of *P. fluorescens* with carbendazim is in agreement with the result of Vidhyasekaran and Muthamilan (1996) and compatability of *P. fluorescens* with mancozeb is in agreement with the works of Mathew(2003), Bhavani (2004) and Paul (2004). Kumar *et al*. (2011) also reported the compatibility of *Bacillus subtilis* MBI 600 (commercial formulation, Integral) with carbendazim and azoxystrobin up to 400 ppm.

Table 3. Compatibility of bacterial antagonists with common agrochemicals against *Xoo* (Interpretation Table)

Sl. No.	Bacterial antagonists& agrochemicals	RR-26	CB-39	RE-1	VB-67	VB-69	RR-53	Pf-1	Chlorpyriphos	Dimethoate	Triazophos	Quinalphos	Dichlorvos	Carbendazim	Mancozeb	Propiconazole	Hexacnazole	Urea	Rajphos	Muriate of potash	Ammonium sulphate
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	RR-26	C																			
2	CB-39	S	C																		
3	RE-1	S	S	C																	
4	VB-67	A	NC	S	C																
5	VB-69	A	S	S	NC	C															
6	RR-53	S	S	S	S	S	C														
7	Pf-1	S	S	S	S	S	S	C													
8	Chlorpyriphos	S	S	S	S	S	S	S	C												
9	Dimethoate	S	S	NC	S	S	S	S	S	C											
10	Triazophos	S	S	S	S	S	NC	S	S	S	C										
11	Quinalphos	**S**	**S**	**S**	**S**	**S**	**S**	**S**	S	S	S	C									
12	Dichlorvos	S	S	S	S	NC	S	S	S	S	S	C	C								
13	Carbendazim	S	S	S	S	S	S	NC	NC	S	S	S	NC	C							
14	Mancozeb	S	S	S	S	S	S	S	S	S	S	S	S	S	C						

The compatibility of *P. fluorescens* with quinalphos and chlorpyriphos is in agreement with the results of Mathew (2003) and Paul (2004). Bhavani (2004) observed that the fertilizers *viz.,* rajphos and muriate of potash were compatible with the antagonists, where as urea, ammonium chloride and ammonium sulphate showed varying levels of inhibition, indicating their partial compatibility.

The compatibility of 13 agrochemicals *viz*., chlorpyriphos, dimethoate, triazophos, quinalphos, dichlorvos, carbendazim, mancozeb, propiconazole, hexaconazole, urea, rajphos, muriate of potash and ammonium sulphate with nine pesticides *viz*., chlorpyriphos, dimethoate, triazophos, quinalphos, dichlorvos, carbendazim, mancozeb, propiconazole and hexaconazole was studied under *in vitro* against *Xoo*. The study revealed that out of 71 two way combinations tested, 19 combinations *viz*., chlorpyriphos with carbendazim, chlorpyriphos with urea, dimethoate with muriate of potash, dimethoate with ammonium sulphate, triazophos with propiconazloe, triazophos with urea, triazophos with muriate of potash, quinalphos with hexaconazole, quinalphos with urea, quinalphos with muriate of potash, dichlorvos with carbendazim, carbendazim with propiconazloe, carbendazim with hexaconazole, carbendazim with urea, carbendazim with rajphos, carbendazim with muriate of potash, carbendazim with ammonium sulphate, hexaconazole with urea and hexaconazole with rajphos showed non compatible effect against *Xoo*. Three two way combinations *viz*., quinalphos with dichlorvos, dichlorvos with urea and hexaconazole with ammonium sulphate showed compatible action against *Xoo* and the rest 50 two way combinations showed synergistic action in inhibiting the pathogen. Manav and Thind (2001) also studied the *in vitro* efficacy of five chemicals against *Xoo* by paper disc diffusion method and found that copper sulphate and monocrotophos could inhibit the bacterial growth.

The compatibility of four fertilizers *viz*., urea, rajphos, muriate of potash and ammonium sulphate showed that, out of six two way combinations, only one namely urea with rajphos showed non compatible action in inhibiting the *Xoo* and the rest five two way combinations showed synergistic action in inhibiting the pathogen. All the seven antagonistic bacteria and 17 agrochemicals showed the compatible reaction in inhibiting the *Xoo*.

It is concluded that except the antagonistic culture VB-67 with VB-69, VB-67 with CB-39 rest of the combinations were showed the synergistic effect in inhibiting the *Xoo*. More over they were found compatible with the most of the commonly used agrochemicals in rice ecosystem. Hence, the promising *Pseudomonas* sp cultures can be made into a microbial consortia for the management of bacterial blight of rice.

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